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RULES AND MECHANISM OF INHIBITION AND CORRELATION IN THE REGENERATION OF *BRYOPHYLLUM CALYCINUM*

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(WITH FORTY-ONE FIGURES)

I. Introduction

In the phenomena of regeneration the problem of correlation appears, that is, the influence of the whole on the part. A part cut out from a whole organism may regenerate, while no such regeneration will occur so long as the part is not separated from the whole. What are the forces inherent in the whole which exercise the control over the part resulting in the prevention of regeneration?

We cannot form a definite idea of this inhibitory mechanism until we know the laws or rules underlying this prevention of regeneration or growth in the normal plant. Only if we succeed in finding such rules and if they are sufficiently simple can we with any hope of success begin to draw conclusions concerning the nature of the mechanism underlying these phenomena of inhibition and correlation. The reason why it is difficult to find such laws lies in the fact that the phenomena of regeneration in most organisms are too complicated or too indeterminate for such a purpose, and we are compelled to look for an organism which is especially favorable for such a purpose. The tropical plant *Bryophyllum calycinum* is apparently such an organism, and the writer has succeeded in finding some rules governing the phenomena of inhibition and correlation of growth. These rules are so simple and transparent that they form, in the opinion of the writer, a securer basis for hypothesis than is offered by most former experiments in this direction which have not led to such simple rules.

The advantage of this plant for the study of the problem of regeneration lies in the fact that shoots can grow out only from definitely located buds in the stem and in the notches of the leaf. The "Anlagen" of roots are not so definitely located, and roots may grow out apparently from practically any spot on the stem of the plant; they are, therefore, not so appropriate for the establishment of definite and simple rules of inhibition, and their growth will not be considered in this paper.

One bud is located in each notch of a leaf of *Bryophyllum calycinum;* when such a notch begins to grow it forms first roots and later shoots. It is well known that if the leaves of this plant are cut off and put on moist soil (or suspended in moist air) they will form roots and shoots from their notches. This is the mode of propagation of this plant. The question is: Why does a leaf not form roots and shoots in its notches so long as it is in connection with a healthy plant? The buds in the notches of the leaf are not the only ones which are inhibited from growing when forming parts of the whole; the buds on the stem, one of which is found in the axilla of each of the two leaves in each node, are in the same condition, and the same question may be raised, namely: Why do not these buds grow out as long as they form part of a plant, while if isolated they may grow into shoots?

A very few words will suffice to show that the stimulus of the wound is not responsible for the growing out of buds, though the conditions at the edge of the wound are responsible for the healing or covering of the wound by the spreading of epithelial cells over the area laid free by the wound; and they may possibly be directly responsible for the callus formation in the case of plants. When we break off a leaf of *Bryophyllum*, the notches of the leaf will grow out into roots and shoots, but these notches are far away from the cut end of the stalk of the leaf. Moreover, as a rule, the notches in the middle of the leaf will grow out first, and not those nearest the wound caused by the cutting or breaking off of the leaf. It is plainly impossible, therefore, to connect in any way the growth of the notches of a leaf with the "stimulus" of the wound. The same may be said for the growth of roots in the main stem of the plant, which may take place several inches away from the seat of injury. We need not dwell on this point any further, since this is generally conceded. It is chiefly in animals that we find regeneration localized at the wound; but this is apparently due to the fact that in such animals any cells may give rise to new growth, while in *Bryophyllum calycinum* the power of giving rise to shoots is restricted to buds located in definite places in the plant.

II. Isolation as the cause of regeneration

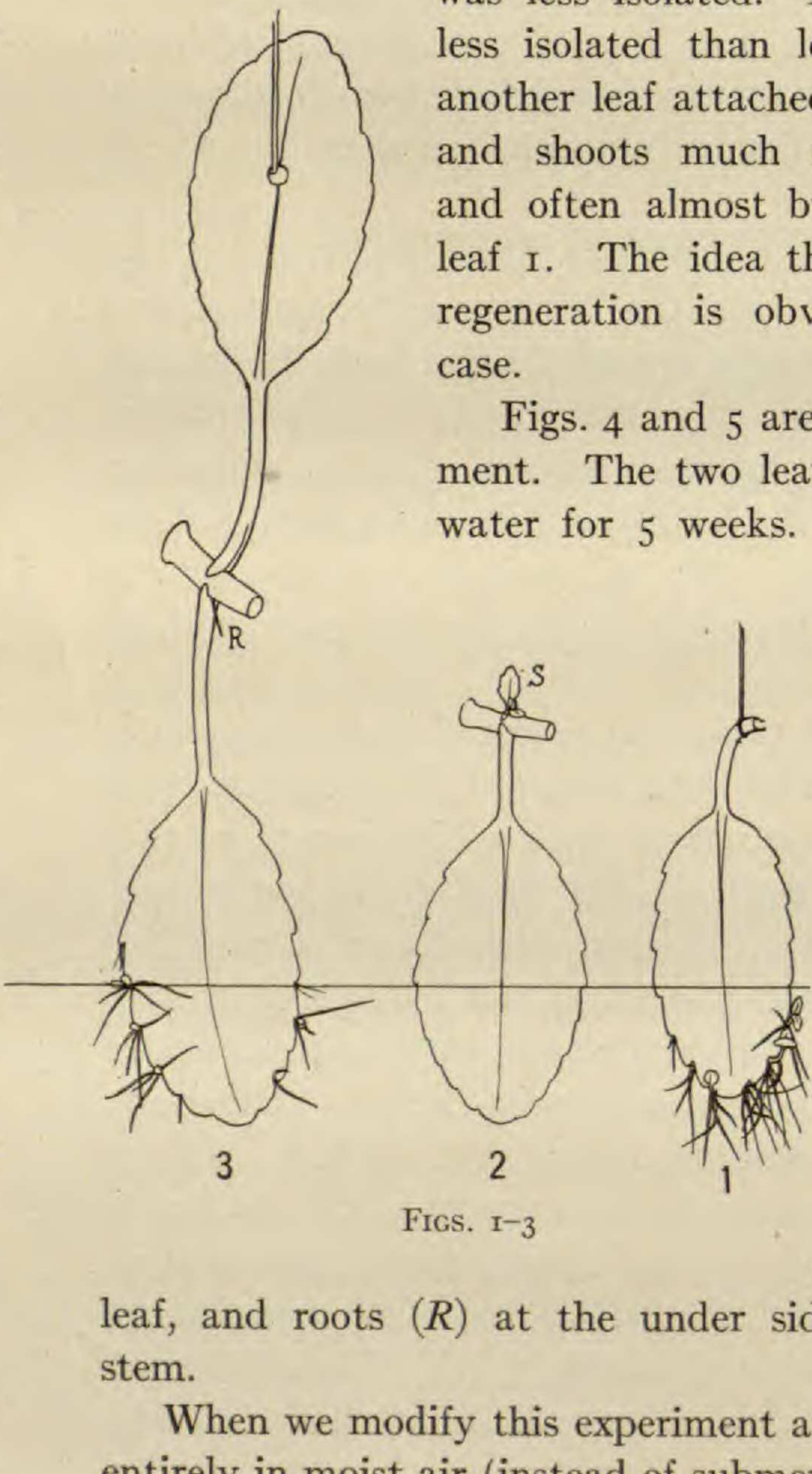
It is generally stated that "isolation" is responsible for regeneration, inasmuch as isolation would release the leaf from the inhibiting influence which the whole has on each part.[1] It is obvious, however, that isolation is an abstract term and that it cannot help us, therefore, in visualizing the forces inhibiting the growth of the buds while the plant is intact. We will show in a simple example that the conception of isolation, while it may fit some cases, will not fit others.

The following experiment was often repeated during the winter months. Three leaves of the same plant of *Bryophyllum* were suspended in an aquarium saturated with water vapor, so that the tips of the leaves (or about one-half of each leaf) were submersed in water at the bottom of the aquarium (figs. 1, 2, 3). Leaf 1 was completely isolated from the stem; leaf 2 had a piece of a stem of the plant attached; and leaf 3 had in addition to a piece of the stem of the plant also the opposite leaf attached. The drawings show the condition of the three leaves after 11 days. Leaf 1 formed roots in a few days, and soon after shoots at the notches of the submersed part of the leaf. In leaf 2, as a rule, all growth from the notches was inhibited, but the bud of the stem opposite the leaf grew out very rapidly into a shoot (fig. 2, *S*). The submersed part of leaf 3 again formed roots and stems in its notches, not quite but almost as quickly as leaf 1. Experiments showed that the

[1] Child, C. M., Die physiologische Isolation von Teilen des Organismus, etc. Roux's Vorträge und Aufsätze. Leipzig. 1911.

result is the same if both leaves of specimen 3 are partly submersed in water; both form roots and shoots in that case.

According to the idea that isolation is the cause of regeneration, we should say that leaf 1 formed new roots and shoots because it was completely isolated; that leaf 2 did not do so (for a long time at least) because, being connected with a piece of a stem, it was less isolated. But leaf 3, which was still less isolated than leaf 2, inasmuch as it had another leaf attached to the stem, formed roots and shoots much more quickly than leaf 2 and often almost but not quite as quickly as leaf 1. The idea that isolation is the cause of regeneration is obviously inadequate in this case.



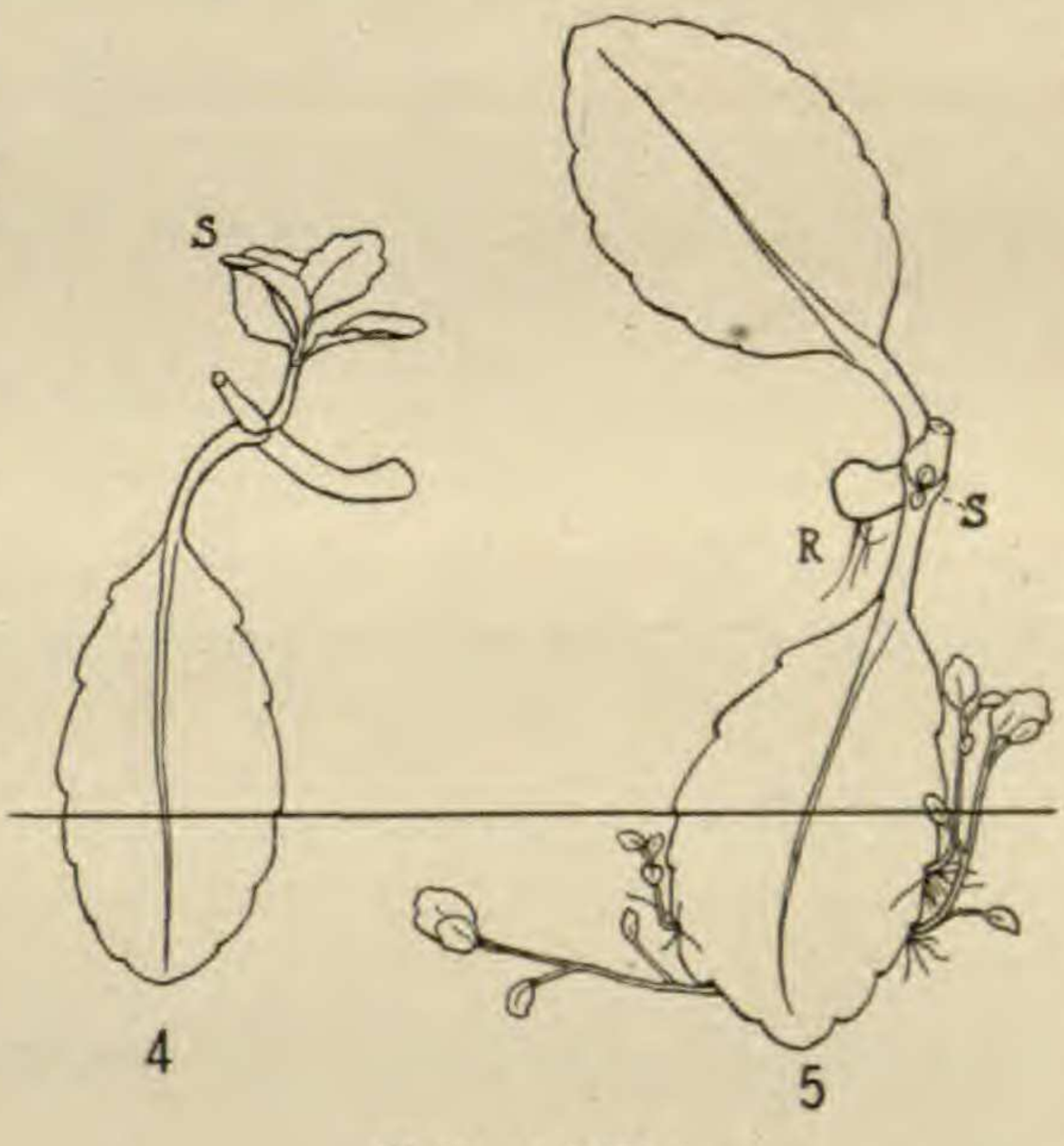
FIGS. 1–3

Figs. 4 and 5 are a repetition of this experiment. The two leaves had been submersed in water for 5 weeks. The leaf in fig. 4, with a piece of stem attached, had formed no roots or shoots in its notches; instead it had formed a long shoot (S) from the bud of the stem opposite the leaf. The leaf in fig. 5, with a piece of stem and the opposite leaf, had formed four shoots from the submersed notches, while the stem had formed one tiny shoot (S) from a bud in the axilla of the lower leaf, and roots (R) at the under side of the basal end of the stem.

When we modify this experiment and suspend the three leaves entirely in moist air (instead of submersing them partly in water), leaf 1 (entirely isolated) will again form roots and shoots in its notches; leaf 2 will as a rule show no growth, but from the opposite bud of its stem a shoot will grow (*S* in figs. 2 and 4); and in the leaves 3 tiny roots may begin to grow from the notches which, however, usually dry up after some time; and no shoots are formed if the leaf is suspended entirely in moist air.

III. Inhibition of growth of leaves by growth of buds on stem

The question now arises: Why does the presence of the piece of main stem in fig. 2 inhibit or retard the formation of roots and shoots in the notches of the leaf, and why does the same piece of stem cease to inhibit (or why does it inhibit considerably less) when, as in fig. 3, in addition to the stem another leaf is left with it? Each node has two buds, one in the axilla of each leaf. When we use a specimen, as fig. 2, a shoot (*S*) will grow out in a few days from that bud of the stem where the leaf is removed; and this is the first growth which will occur in this specimen. The bud in the axilla of the leaf which is preserved will as a rule not grow out. In fig. 3, where both leaves are preserved, neither bud of the stem will grow out in winter.[2] Hence we notice that where a shoot grows out very rapidly from the bud of the stem, as in fig. 2, the leaf in contact with the stem is prevented or delayed in forming roots and shoots, but when no such shoots grow out from the bud of the stem (as in fig. 3), the notches of the leaf (if submersed in water) will form roots and shoots rather quickly.



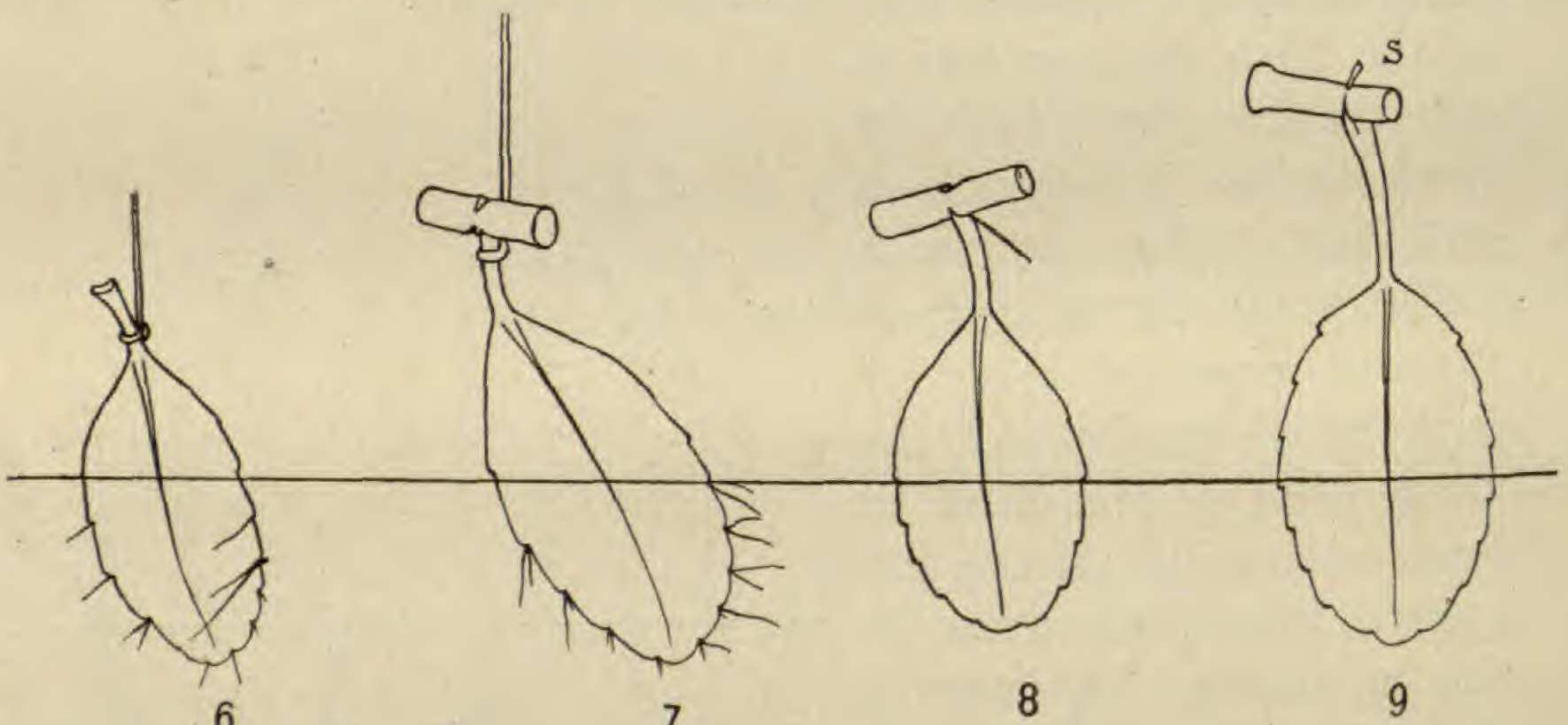
FIGS. 4 AND 5

[2] In the spring this is not so strictly true, but all these experiments were made in a greenhouse during the winter months. The greenhouse had a temperature of 70° F. or above.

The stalk of an isolated leaf without any piece of the stem is not capable of giving rise to any regeneration. Such a leaf will form adventitious roots and shoots in its notches very rapidly.

All these facts make it appear as if the growth of the buds on a piece of the stem might have an inhibiting influence on the growth of the adventitious roots or shoots of the leaf.

In order to estimate properly such an influence, an extensive series of experiments was made, in which leaves with a piece of stem attached were submersed with their tips in water, while the rest of the specimen was in moist air. In a number of stems both buds were removed (fig. 7), in another only the upper buds were



FIGS. 6–9

removed (fig. 8), while in the rest of the stems none of the buds were removed (fig. 9). If the inhibiting effect of the stem were *exclusively* due to the growth of the buds on the stem, the latter should lose its inhibiting effect entirely if these buds were removed; and the leaf connected with such a "debudded" stem should form adventitious roots or shoots as fast as a leaf without any stem. This was, however, not entirely the case. While a leaf connected with a "debudded" stem (fig. 7) formed as a rule its adventitious roots more quickly than a leaf with a normal stem (fig. 9), the leaves connected with the "debudded" stem formed their adventitious roots not quite so quickly as the completely isolated leaves (fig. 6).

Figs. 6–10 give the average results of such experiments. The drawings were made May 10, seven days after the operation. The isolated leaves (fig. 6) had all formed their adventitious roots, and so had some but not all the leaves with "debudded" stems (fig. 7). The leaves which had lost only the upper bud had not formed roots as fast as the leaves with entirely "debudded" stems (fig. 8). The leaves with normal stems (fig. 9) had not yet formed any adventitious roots, but the shoot (*S*) on the stem where the leaf had been removed had begun to grow out.

The following record of an experiment performed May 1 had yielded on May 10 the following result:

1. Completely isolated leaves (fig. 6). All ten leaves had formed adventitious roots and tiny shoots.

2. Eighteen leaves each attached to a completely "debudded" stem (fig. 7). Eleven leaves had formed adventitious roots and one also adventitious shoots.

3. Ten leaves with a stem whose upper bud was removed (fig. 8). Four leaves had formed adventitious roots or shoots.

4. Ten leaves with a normal stem (fig. 9). All these stems formed shoots from the upper bud. No leaf has formed adventitious roots or shoots.

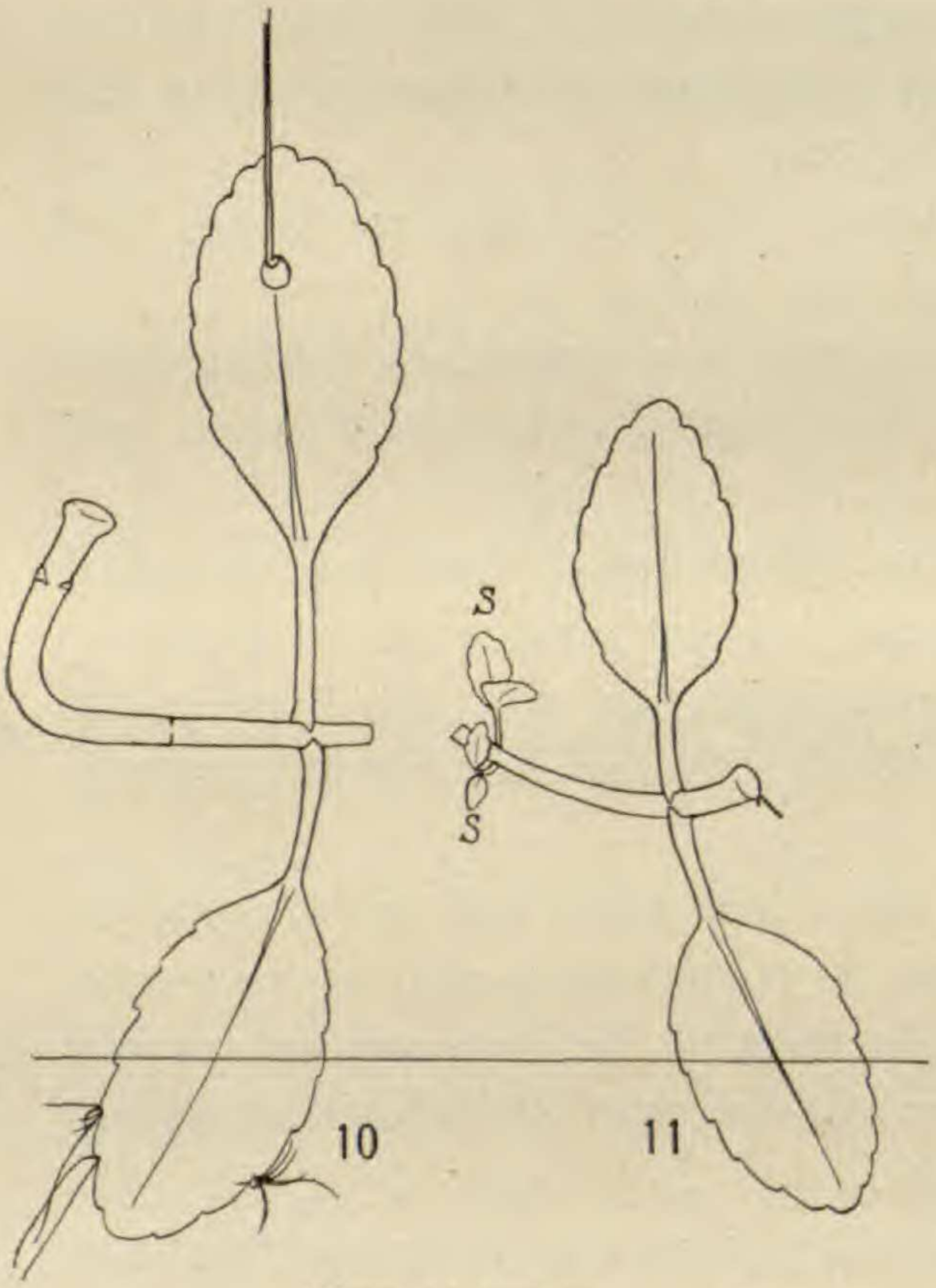
It is, therefore, obvious first that a stem whose buds are removed has still an inhibiting influence upon the formation of roots in the notches of a leaf; and second, that if the buds of the stem are not removed the growth of the bud opposite the leaf enhances this inhibiting effect of the stem upon the leaf considerably.

Since the growth of this bud of the stem is as a rule also inhibited when the opposite leaf is not removed, as in fig. 3, we understand why the non-removal of this leaf favors the growth of the adventitious roots from the notches of the other leaf.

We have seen that isolated leaves when suspended *in moist air* will form roots and shoots from their notches even if they are not submersed in water; while if a leaf is connected with a stem, the formation of roots and shoots in the notches will be permanently inhibited *in moist air*. It should be added, that the leaves attached to a "debudded" stem may form very short adventitious roots when suspended in moist air (instead of in water), but will not form long roots or shoots as will the completely isolated leaf. The analogy between the effect of the non-removal of the opposite leaf from the stem and of the removal of the opposite bud seems thus pretty complete.

IV. Continuation of these experiments

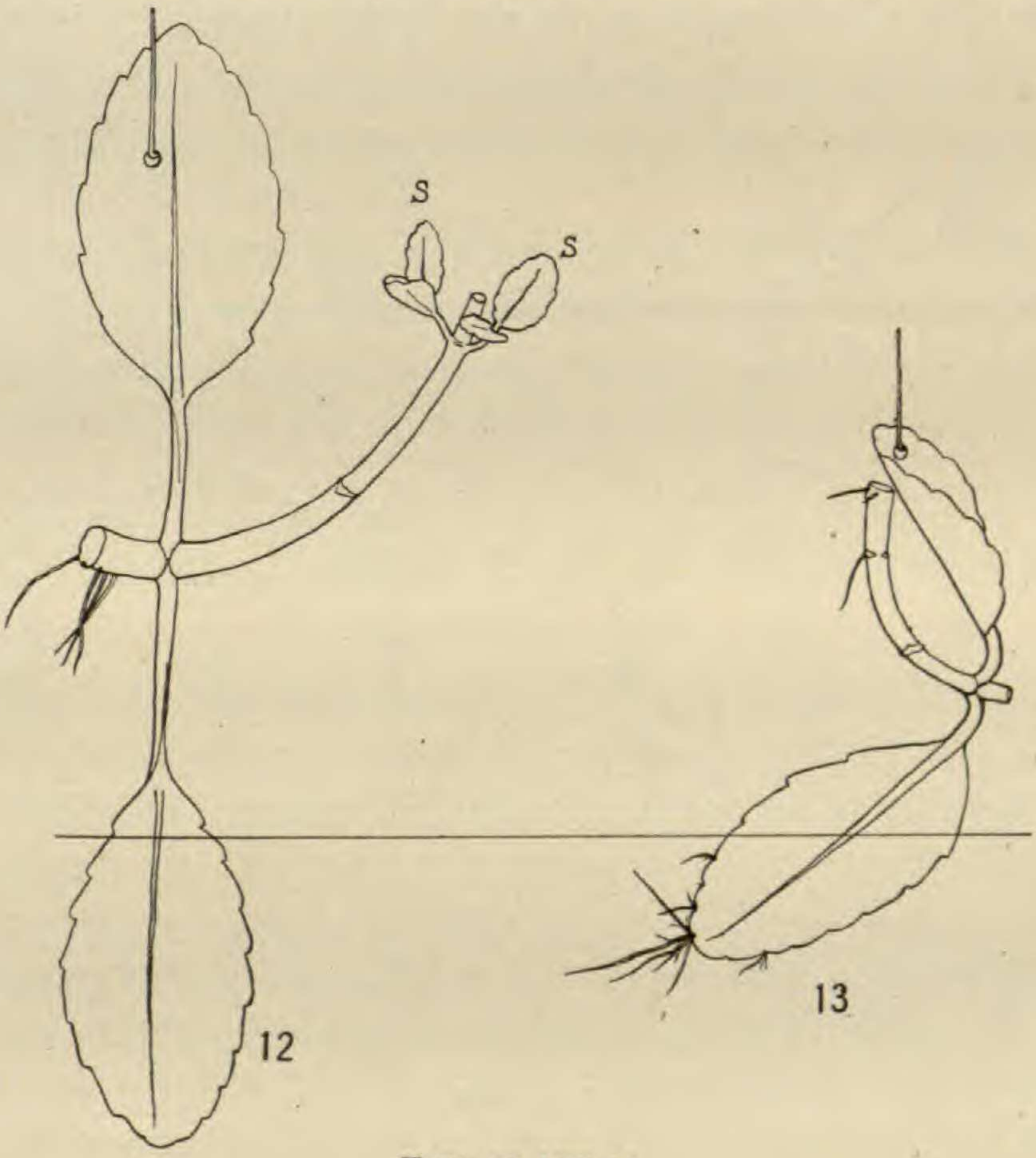
We have thus seen that the growth of buds on the stem is one factor which inhibits or delays the growth of the notches in the opposite leaf. We intend to show the influence of this factor in some further observations.



FIGS. 10 AND 11

In all previous experiments we had cut out from a plant a piece of stem with only one node. If we cut out a piece of a stem containing two or three nodes (figs. 10, 11, 12, 13) and preserve one pair of leaves, the behavior of these leaves will be different if they are left in the apical or in the basal node of the piece. Figs. 10–13 illustrate this difference. In all cases one or both leaves are partly submersed in water, while the rest of the preparation is suspended in moist air. In such cases new shoots (*SS*) were formed in a few days from the two apical buds of the stem in fig. 11, where the apical leaves had been removed and only the basal leaves left; while in specimens like fig. 10, where the apical leaves were left, the buds on the stem either formed no new shoots or formed them with some delay. As a consequence, we notice that in fig. 11 the submersed leaf formed at first no shoots, while the submersed leaf in fig. 10 in about 50 per cent of the cases formed roots and shoots in its notches rather rapidly. This happened often, but not always, when the formation of shoots on the stem itself was long delayed. Ultimately all the leaves may form adventitious roots and shoots in the notches that are under water or near the edge of the water.

This experiment, therefore, supports the conclusion that if the buds of the stem grow out very rapidly, their growth inhibits or

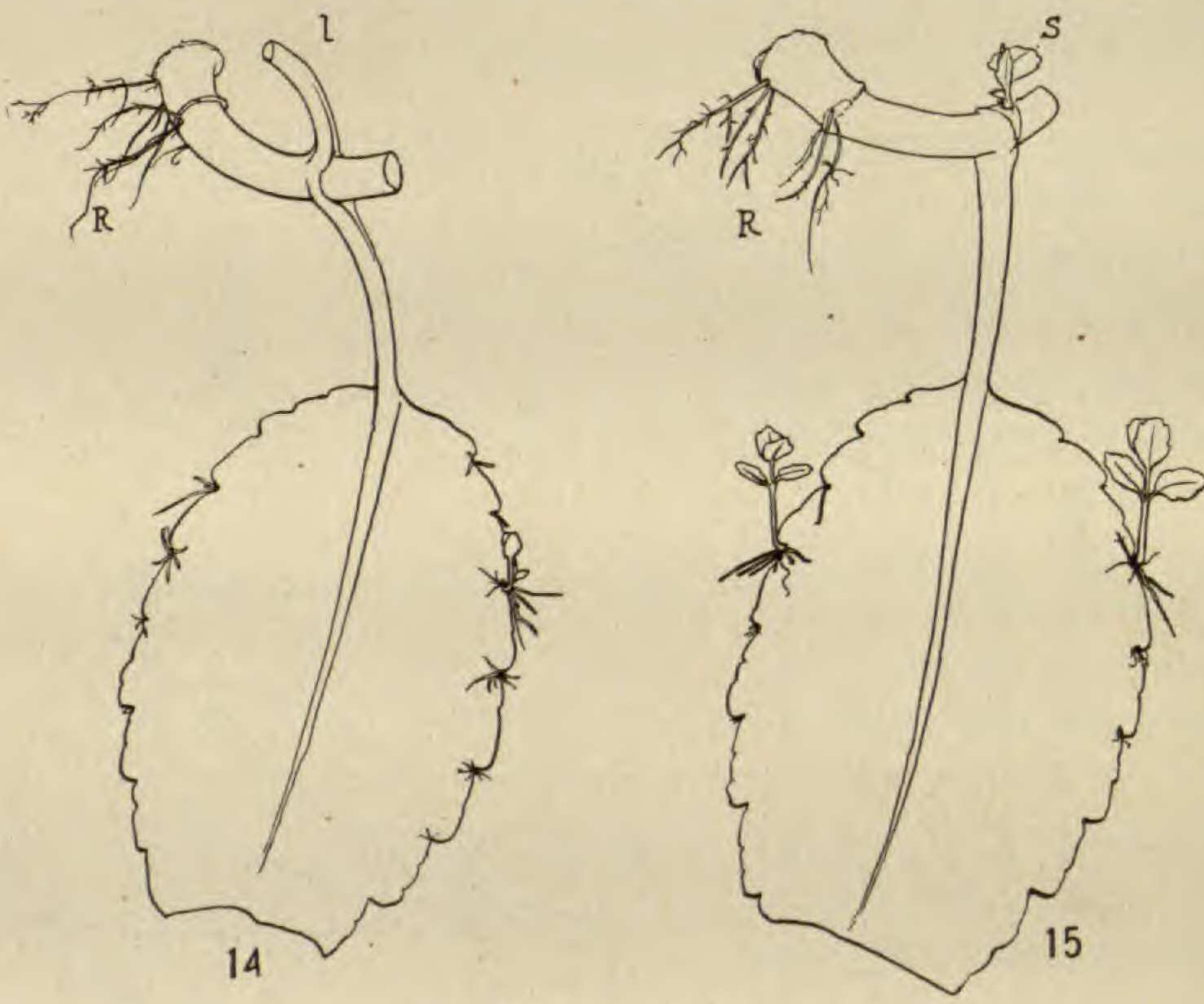


Figs. 12 and 13

delays the growth of roots and shoots in the notches of the leaf attached to the stem.

Figs. 12 and 13 are a repetition of the same experiment. The drawing was made 17 days after the beginning of the experiment. The stem in fig. 12 formed rapidly two shoots (*SS*) from its apical buds and this inhibited the growth of roots and shoots in the submersed leaf; in fig. 13 the stem formed no shoots and the submersed leaf could form roots. The root formation in both stems was about equal. These experiments have been repeated so often that they can be asserted to form reliable demonstration experiments, during the winter months at least.

We have already stated that in a completely isolated leaf (as in fig. 1) the roots in the notches in the leaf do not begin to grow until a few days after the bud in a stem (*S* in fig. 2) has begun to grow. It would seem, therefore, that we might weaken the inhibiting influence of a piece of stem, as shown in the experiment in fig. 2, if



FIGS. 14 AND 15

we inhibit or retard the shoot formation of the bud on the stem. This can be done, as we have already stated, by not removing the other leaf on the stem, as in fig. 3. It is not necessary, however, to leave the whole leaf attached to the stem; it suffices if we leave a piece of the stalk of a leaf attached as in fig. 14. In this case a leaf (with a piece of stem and a piece of the stalk *l* of the other leaf) were suspended November 12 in moist air. The bud in the axilla of the stalk *l* was by the presence of the latter prevented from growing out, and after some time roots (*R*) were formed at the basal end of the stem. Still later, roots and shoots began to grow out from the notches of the leaf (although this was not submersed in water). Fig. 14 was drawn January 18, therefore 9 weeks after the beginning of the experiment. About a week after the drawing was made, the stalk *l* which had wilted fell off and now the bud in the axilla of the stalk *l* was able to grow and the shoot *S* was formed (fig. 14). The drawing (fig. 15) was made when the shoot was one week old. The experiment shows also incidentally that the *root* formation on the stem does not (under the conditions of this experiment) inhibit the formation of roots and shoots on the leaf. We shall return to this fact later.

In this experiment the leaf was merely suspended in moist air and yet shoots developed from the leaf although it was attached to a piece of stem. This is unusual, since in order to obtain such a result with certainty it is necessary to submerse part of the leaf in water.

V. Inhibiting influence of roots on the growth of the notches of a leaf

A piece of stem when cut from a whole plant of *Bryophyllum* is not only able to form shoots but it also forms roots, and it is now our intention to consider the influence which the root formation of the stem has on the growth of the notches of a leaf. WAKKER, DEVRIES,[3] and GOEBEL[4] all have reached the conclusion that it is the presence of the main root or the regenerated roots on the stem which prevent the growth of adventitious roots or shoots on the leaf. If we break or cut off a leaf of *Bryophyllum calycinum* from the stem, neither the stalk nor the base of the isolated leaf has the power of forming roots, and this inability of root formation is considered by WAKKER and DEVRIES to be the cause of the growth of the notches. "According to WAKKER the organic separation of the leaf from the rooted part of the plant acts as a stimulus upon the leaf and induces the growth in the notches."[5]

[3] DEVRIES, HUGO, Jahrb. Wiss. Bot. 22:35. 1890.

[4] GOEBEL, K., Einführung in die Morphologie der Pflanzen. Leipzig, 1908 (pp. 142–149).

[5] DEVRIES, HUGO, *loc. cit.* The writer was not able to obtain WAKKER's monograph.

DeVries describes a very striking experiment which supports the idea of Wakker that the root of the main plant is the factor which inhibits the growth of the notches in the normal plant.

The apices of six plants were cut off beneath the most vigorous adult leaf and planted in soil. After strong roots had been formed (in the soil) their stems were cut above the lowest pair of leaves, the apices removed, and this lowest pair selected for the experiment. Both leaves were put flatly on moist sand, the one after having been removed from the stem, while the other remained connected with the roots. The axillary buds were destroyed. After three weeks the isolated leaves had formed numerous young plants on their margin. The leaves which had remained connected with the rooted piece of stem had formed no plants in their notches (and did not form any afterward), although they had otherwise been exposed to the same conditions as the isolated leaves.

This experiment is in harmony with the view that the normal roots of a stem (if they are under normal conditions) inhibit the growth of notches in the leaves. DeVries reports a second experiment in favor of the view of Wakker. He cut the stem of a plant in its internodes and thus isolated seven pairs of leaves.

From each pair one leaf was broken off; all axillary buds were destroyed. The leaves were now put on moist sand. After a month the seven stems had formed roots. The isolated leaves[6] had formed in their notches rooted plantlets, varying from 10 to 26 in number. The leaves whose stems had formed roots behaved differently. One leaf had formed no trace of growth in its notches; it was the one whose stem had formed roots first. The rest of the leaves had formed only a few plants whose number varied between 2 and 6. They reached only a few mm. in length, while those of the isolated leaves measured from 0.5 to 2 cm.

We see here that if a root is formed on a stem before the roots in the notches of a leaf can grow out, the root (under proper conditions) may inhibit the growth of the notches of a leaf.

While these facts leave no doubt that the root (under proper conditions) can inhibit the growth of the notches in the leaves of *Bryophyllum*, the experiments mentioned on the previous pages of this paper show that this is not the only factor. A piece of stem, even if it does not form any roots but only a shoot, will inhibit or greatly delay the growth of the notches of a leaf connected with it.

[6] That is, those broken off from the stem.

VI. Influence of root formation and of root pressure

DeVries assumes with Wakker that it is not the root formation in itself by which the stem or main plant inhibits the growth in the notches of a leaf, but the root pressure. Goebel is inclined to think that it is the root formation in itself, regardless of the root pressure (or flow of water caused by it), which inhibits the growth in the notches of the leaf. The writer has made a number of observations which indicate that of the two views that of Wakker and DeVries is better supported by the facts.

As an illustration, we may take figs. 14 and 15, in which a leaf with a piece of stem was suspended in moist air. The basal end of the stem formed a mass of roots, yet this did not prevent the growth of roots and shoots from the notches of the leaf. This contradicts Goebel's assumption, but is in harmony with the view of DeVries, since these roots in the air were not able to give rise to "root pressure."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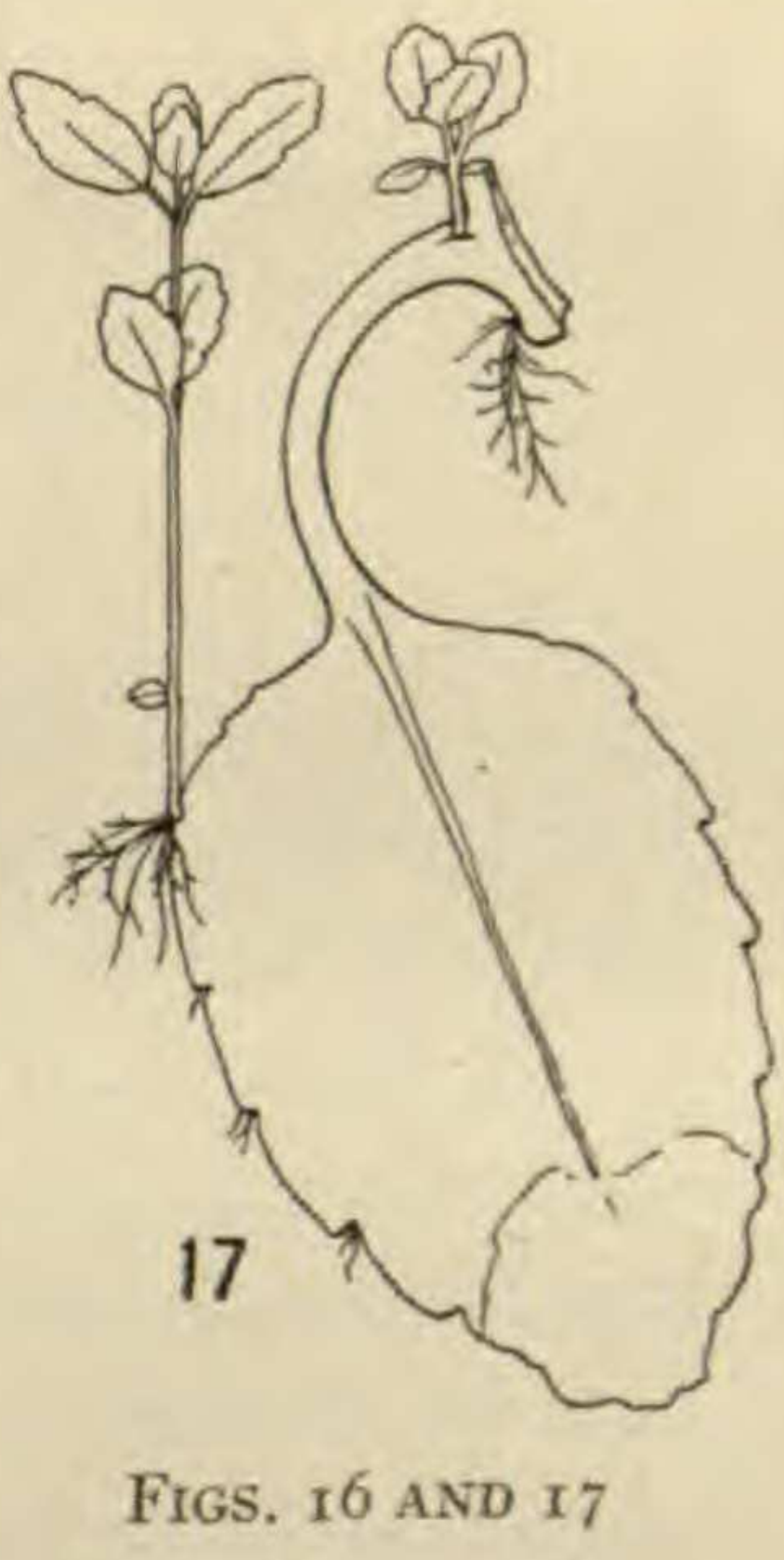
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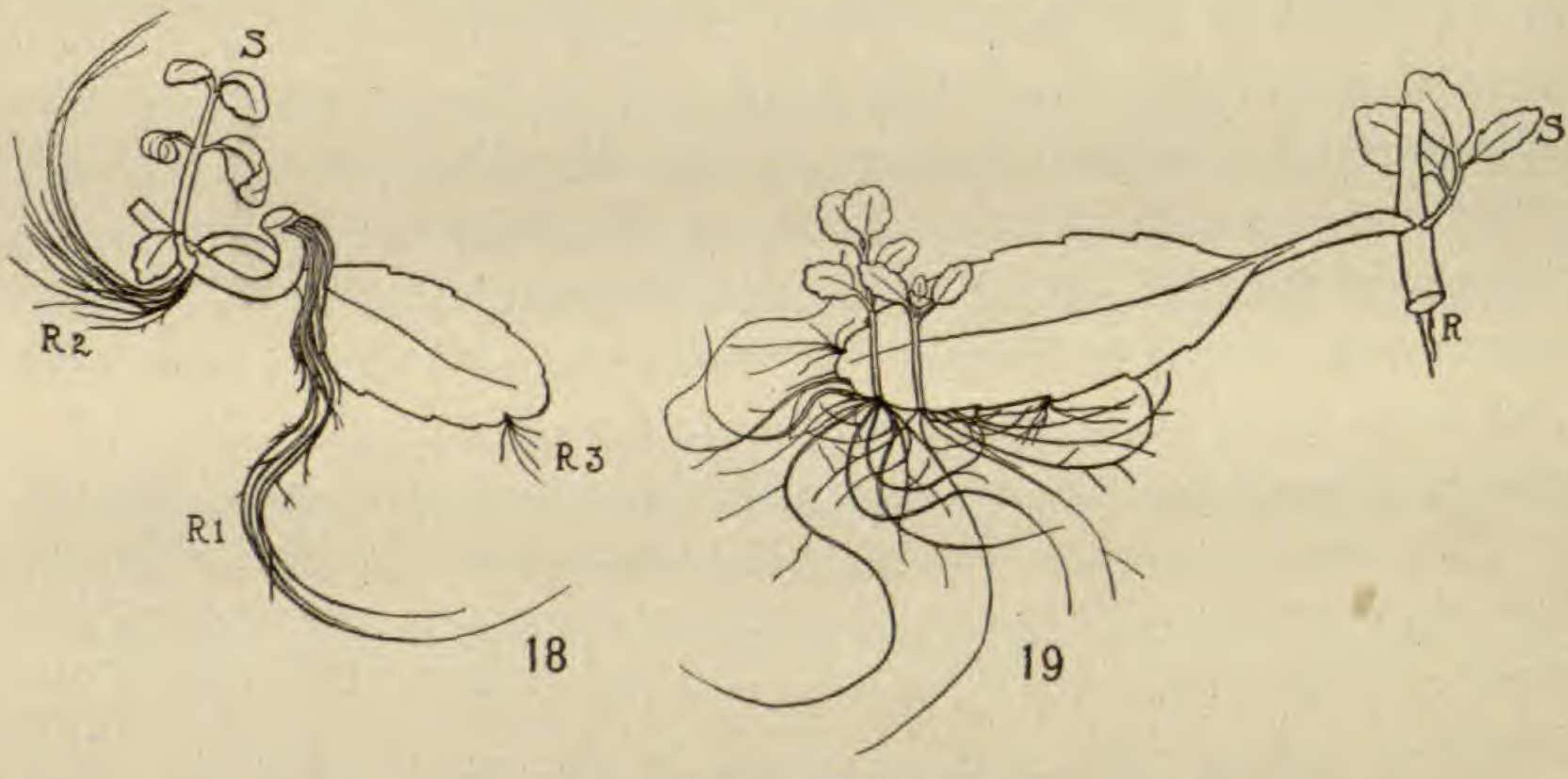
Figs. 16 and 17

As a further support, we may give the drawings (figs. 16 and 17). In these cases the leaves were cut off with only a fragment of the stem attached, which was a little larger in fig. 17 than in fig. 16. The axillary bud was not removed. The leaves were suspended in moist air. Although the remnants of the stems formed roots, yet the leaves formed also roots and shoots (although they were in moist air). The root formation on the remnant of the stem preceded the root formation on the leaf but did not prevent the latter. The experiment shows again that mere root formation in a stem suspended in moist air does not prevent the formation of roots and shoots in a leaf of *Bryophyllum*. It should be pointed out, however, that the shoots grew out from the axillary bud of the leaf; it is the growth of the opposite bud which has the inhibitory power on the leaf mentioned in the third and fourth sections.

While the root formation on the stem does not inhibit the shoot formation on the leaf if the root is exposed to moist air, the result is different if the roots are in water. If one leaf with a piece of stem (fig. 18) is put into a Petri dish, the bottom of which contains a thin layer of water, the stem will form enormous roots (*R*1) at its basal end from the callus, and *R*2 from the basal end of the shoot (*S*) which grows out from the bud of the stem where the leaf was removed. On the other hand, the leaf has formed only a few small roots (*R*3) at one notch. (As a rule the notches of the leaf formed no roots in such an experiment.) In this case the roots of the stem which were functioning, and probably established the



FIGS. 18 AND 19

usual root pressure, inhibited for a long time and in most cases permanently the regeneration in the leaf.

If, however, we do not put the leaf immediately after it is cut from the plant into the Petri dish but suspend it first in moist air, the stem will form a shoot (*S* in fig. 19). Roots (*R*) may or may not be formed on the stem, but they will always be formed considerably later than the shoot, at least in winter. If after a month we put the leaf into a Petri dish, while the stem remains in moist air, the leaf (fig. 19) will rapidly form roots and shoots. The contrast between the behavior of the leaves in this case and the one mentioned before is very striking. In the experiment represented in fig. 19, the roots (*R*) at the base of the stem could not establish a flow of water in the stem and could not inhibit the growth of the shoots in the notches of the leaf; and by the time the leaf was put in water they were obviously not in a position to produce a flow or a root pressure.

Roots formed on the stem have as a rule, therefore, an inhibiting effect on the growth from a leaf if they can produce a root pressure, that is, if they are in water. The formation of a shoot from the bud of a stem can produce such an inhibiting effect upon the opposite leaf if the shoot is in moist air and if no root is formed. This influence of roots on the growth of the notches of a leaf was discussed only for the sake of completeness, since it does not strictly belong to our problem, which deals only with the growth of shoots.

VII. The conditions inhibiting and accelerating the growth of the axillary buds

Each node of *Bryophyllum* has one pair of leaves, and in the axilla of each leaf is found a bud which in the normal life never grows out, but which may grow out as a consequence of a mutilation of the plant.

If we cut through two successive internodes of a stem and isolate a single node, and if we remove the two leaves, the two buds on the stem will grow out rapidly (if we provide the necessary water supply or if the node was cut out from near the base of the stem).

If we remove only one instead of both leaves, only one bud will as a rule begin to grow, namely the one whose leaf is removed. This suggests the idea that the leaf, while favoring the growth of the opposite bud, inhibits the growth of its own axillary bud. If we remove neither of the two leaves, in many cases (especially in winter) neither bud will grow out, a fact which harmonizes with the assumption that each leaf suppresses the growth of its own axillary bud.

The following experiment, however, restricts this last assumption that each leaf will inhibit the growth of its axillary bud. If we isolate a node with its two leaves (which we do *not* remove), and if we split the piece of stem longitudinally, we obtain two leaves, each attached to a half of a node containing the axillary bud of the leaf (fig. 20). In this case the axillary bud will grow out, although often with some delay. Hence the leaf in this case does not prevent the growth of its own axillary bud, and if we speak of an inhibition in the previously mentioned cases, we have to add the remark that this inhibition only exists if the other leaf or the opposite bud are in connection with the first leaf. A comparison of figs. 20 and 21 is of interest. In both cases leaves with a piece of stem attached were suspended in moist air on February 20. They were drawn on April 1. In fig. 20 the shoot (*S*) in the axilla of the leaf left attached to a longitudinally split piece of stem grew out. In fig. 21, one leaf with a whole, non-split piece of stem attached gave rise to the growth of the shoot (*S*) on the upper side of the stem where the leaf was removed, while the bud in the axilla of the leaf was prevented from growing.

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FIGS. 20–22

Fig. 22 is a case similar to fig. 14, one leaf with a piece of stem and the stalk *l* of the opposite leaf. In this case the bud (*S*) in the axilla of the intact leaf grew out. This is not the most common experience. More often in winter neither of the two axillary buds of the stem will grow out under such conditions. The experiment in which the piece of stem is split longitudinally (fig. 20), however, generally succeeds.

The following observation is also of some significance. If we cut out a node, remove one leaf and its bud, but preserve one leaf and the bud in its axilla, the latter will grow out into a shoot after some delay. Hence the removal of the opposite bud removes the inhibiting influences which this bud naturally has on the growth of the bud in the axilla of a leaf. We can accelerate the growth of this latter bud, however, when in addition to the removal of the opposite bud and leaf we make an incision or cut out a piece from the rind apically to the axillary bud whose leaf is not removed. In this case the bud in the axilla of a leaf which is not removed will grow out rather rapidly.

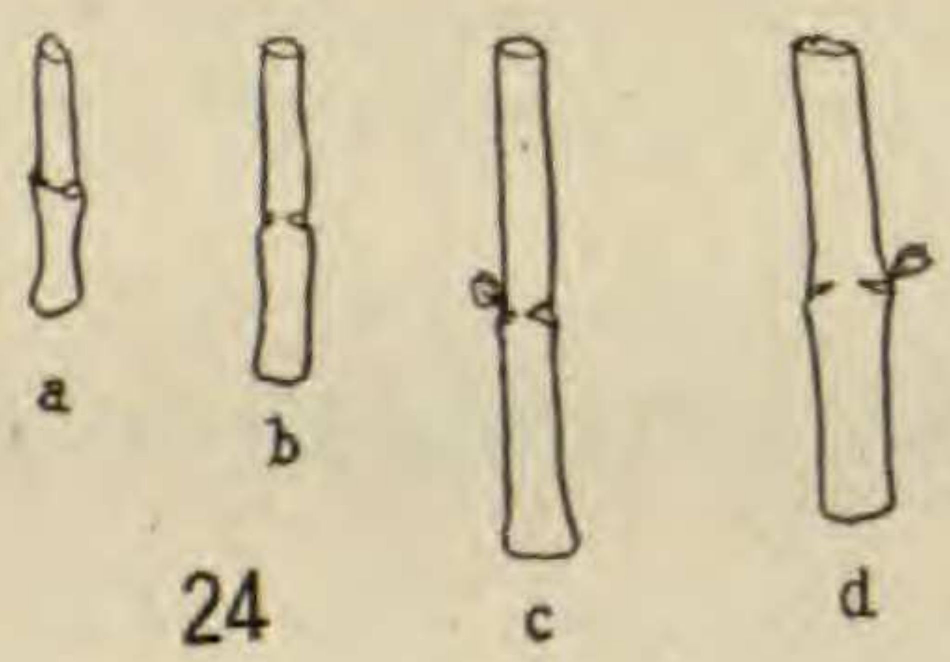
We may anticipate that all these experiments indicate that the growth of the bud depends upon the flow of certain substances from the leaf to the bud. That bud which receives these substances first will grow out first, and thereby prevent the flow to the other bud whose growth is thereby "inhibited." The apparent inhibition of growth in one place is simply due to the fact that under the conditions of the experiment the substances required for growth flow to some other place and are retained there, and the removal of the inhibition consists in creating conditions which will force the substances to flow where we want growth to occur.

VIII. The rules and mechanism of inhibition in regeneration

We cut off the base and tip of the main stem of a plant of *Bryophyllum*, remove all the leaves, and suspend the stem in a closed aquarium saturated with water vapor. Only the two buds at the highest apical node will grow out (fig. 23); it does not matter whether the stem is hung upright or inverted. The buds at the more basal nodes are all inhibited from growing by the growth of the two apical buds; for if we isolate any of the lower nodes, their buds also may grow (fig. 24). This is the well known example of an inhibition of one part by another. In the terminology of REINKE, we might call the two apical buds the "dominants." What is the source of their dominance? By way of an answer we intend to show that the following relation exists: *If an element a inhibits the growth in an element b, b very often accelerates or makes possible the growth in a.* When we cut off a single node near the top of the main stem of *Bryophyllum*, remove the two leaves, and suspend it in an aquarium saturated with water vapor, as a rule the two buds will not grow out. If, however, we leave it in connection with one or more of the lower nodes of the stem, it will regenerate, and incidentally inhibit the growth in the lower nodes (figs. 23, 24). The regeneration and growth of the two shoots at the apical node will as a rule be the quicker the more nodes are left in contact with it. Hence the lower part of the stem whose regeneration is inhibited by the apical node, at the same time accelerates the latter's regeneration or makes it possible.

The second example is the following: When we cut off one leaf with a piece of the main stem (as in fig. 2) and suspend it in water, the bud opposite the intact leaf will grow out into a shoot (*S* in fig. 2). We have seen that the growth of this shoot has a share in the inhibition of the growth of the notches of the leaf in this experiment. It can be shown that conversely the leaf accelerates or renders possible the growth of the bud in the stem. As stated, the isolated node near the top will not be able to form shoots if suspended in moist air. If, however, one leaf or even a fraction of one leaf is left in connection with the stem, the bud on the opposite side will grow out (figs. 25–29). In the isolated nodes (figs. 28, 29) cut off near the apex no buds could grow in moist air. When, however, only a piece of a leaf was left in connection with such a stem, as in fig. 25 or 26, the bud could grow out. Here we see again that the presence of the leaf accelerates or renders possible the growth of that shoot in a stem whose formation inhibits or retards the growth of the notches of the leaf.


23



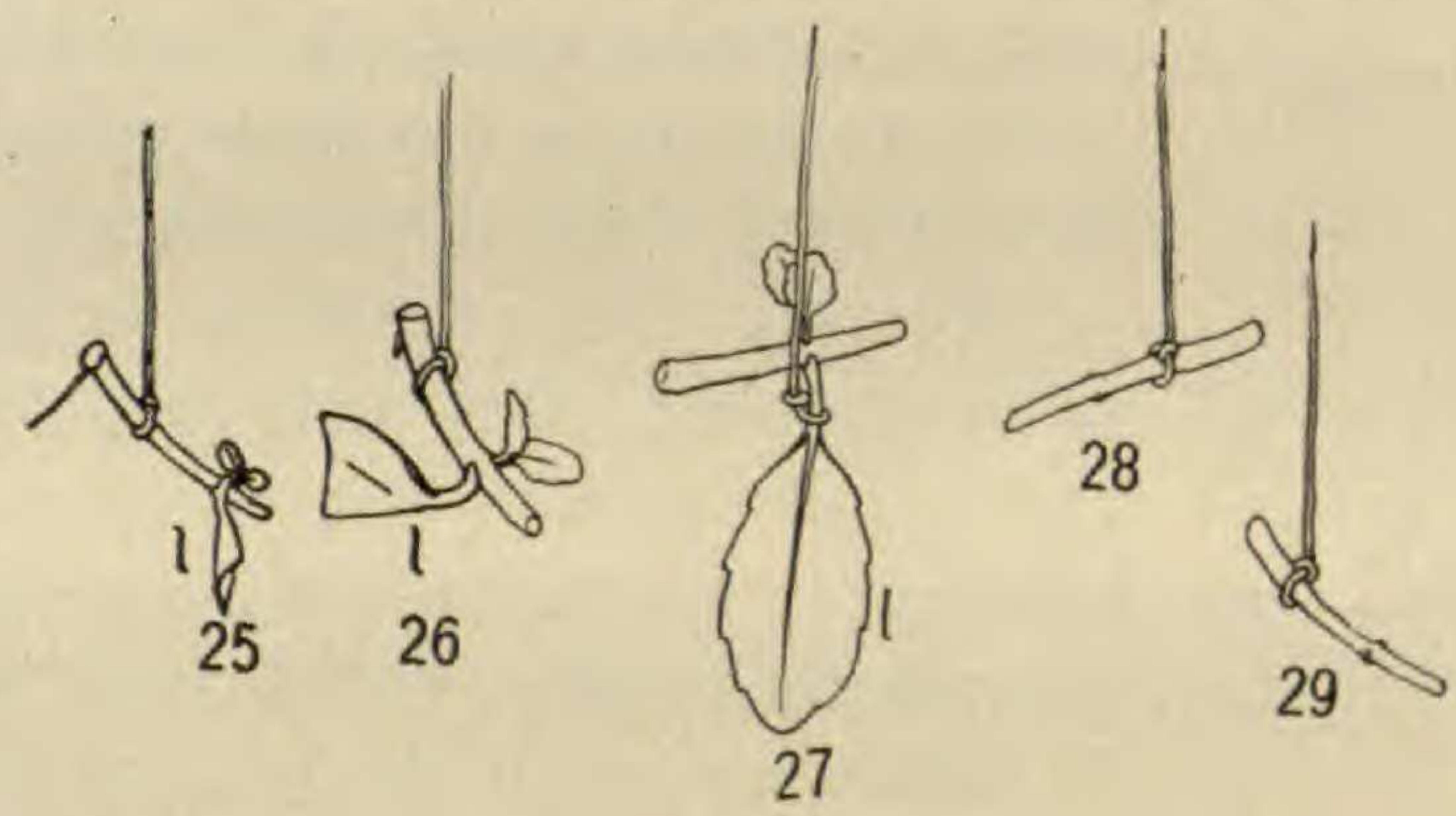
FIGS. 23 AND 24

This gives us a clue to the nature of the dominance and the power of inhibition. The inhibition seems to consist in this, that the dominant part receives something from the inhibited part which accelerates growth or renders it possible in the former.

When we put an isolated node from near the top (whose leaves are removed and which cannot regenerate in moist air) in a very thin layer of water, new shoots grow out (figs. 30–32). This looks as if the "something" which the inhibited part supplies to the dominant part were water. But the writer is suspicious that the water may be only indirectly needed, namely to render the flow of material in the conducting vessels possible. In animals we know that the blood vessels must be filled to render a closed circulation possible. It would seem as if in plants a flow of substance through conducting vessels should be possible only if a certain minimum amount of water is contained in the conducting cells or vessels.

The buds of an isolated node nearer the base of the stem may grow out if suspended in moist air, probably because such a piece does not dry out so easily.

The following experiment rarely fails. If we suspend a piece of stem consisting of several nodes and stripped of all leaves in moist air (fig. 33), the two most apical buds (*b*) will grow out. Their growth, which is usually slow, is greatly accelerated if we leave one leaf (or more) on the stem (*b* in fig. 34). In two weeks the growth of the apical buds (*b*) in fig. 33, which had no leaves, was very slight, while it was strong in the stem (fig. 34) in which one



FIGS. 25–29

leaf was left. Here we have the combined accelerating effect of stem and leaf upon the growth of the apical bud.

Why is it that the apical bud grows out first? Should this be connected with the anatomy of the conducting vessels, possibly in the way that the majority of these vessels go directly from the leaves to the growing point at the apex?

Since the rapid growth of the bud on a stem inhibits or retards the growth of adventitious roots of the opposite leaf (fig. 2), it follows that the removal of the bud or the inhibition of its growth should favor the growth of adventitious roots in the notches of the leaf. This is indeed the case. If we suppress the growth of the two buds in an isolated node, we favor the growth of adventitious roots in the leaves if they are submersed in water (fig. 3). The same happens if we split the node longitudinally (figs. 35, 36). The leaf (fig. 35) connected with a longitudinal half of a node was submersed in water and formed adventitious roots in nine days. The leaf (fig. 36) attached to a whole node formed no adventitious roots under the same conditions.

IX. Isolation, inhibition, and the flow of material through the plant

These rules give us some basis on which we may try to form a preliminary idea on the nature of the mechanism of inhibition. As we mentioned already, the rules are comprehensible if we assume a flow of certain (possibly specific) substances (or formed cells) from the places where the dormant buds are ready to grow, or the prevention of such a flow toward these dormant buds.

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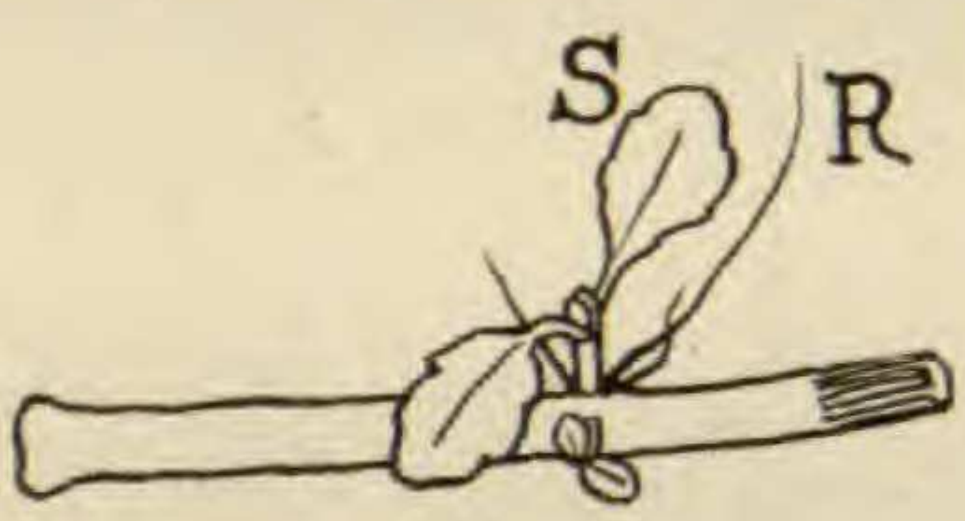

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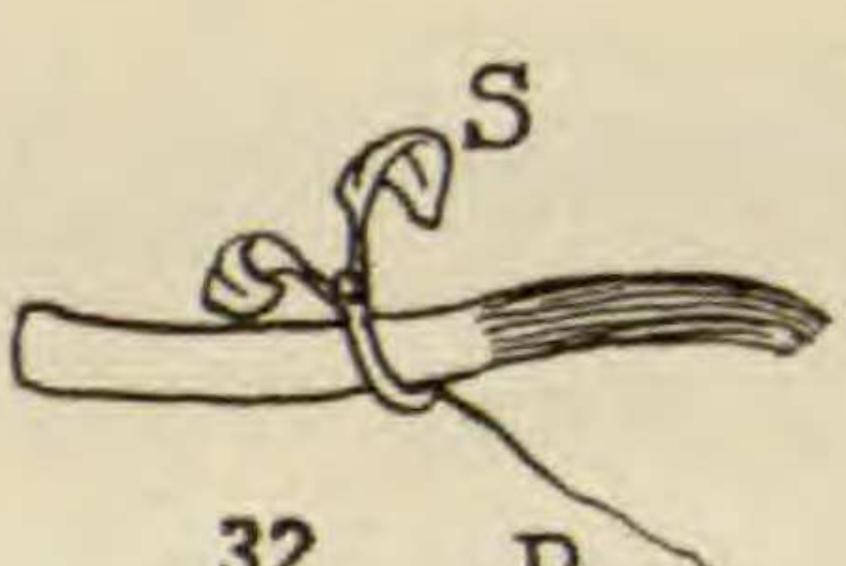


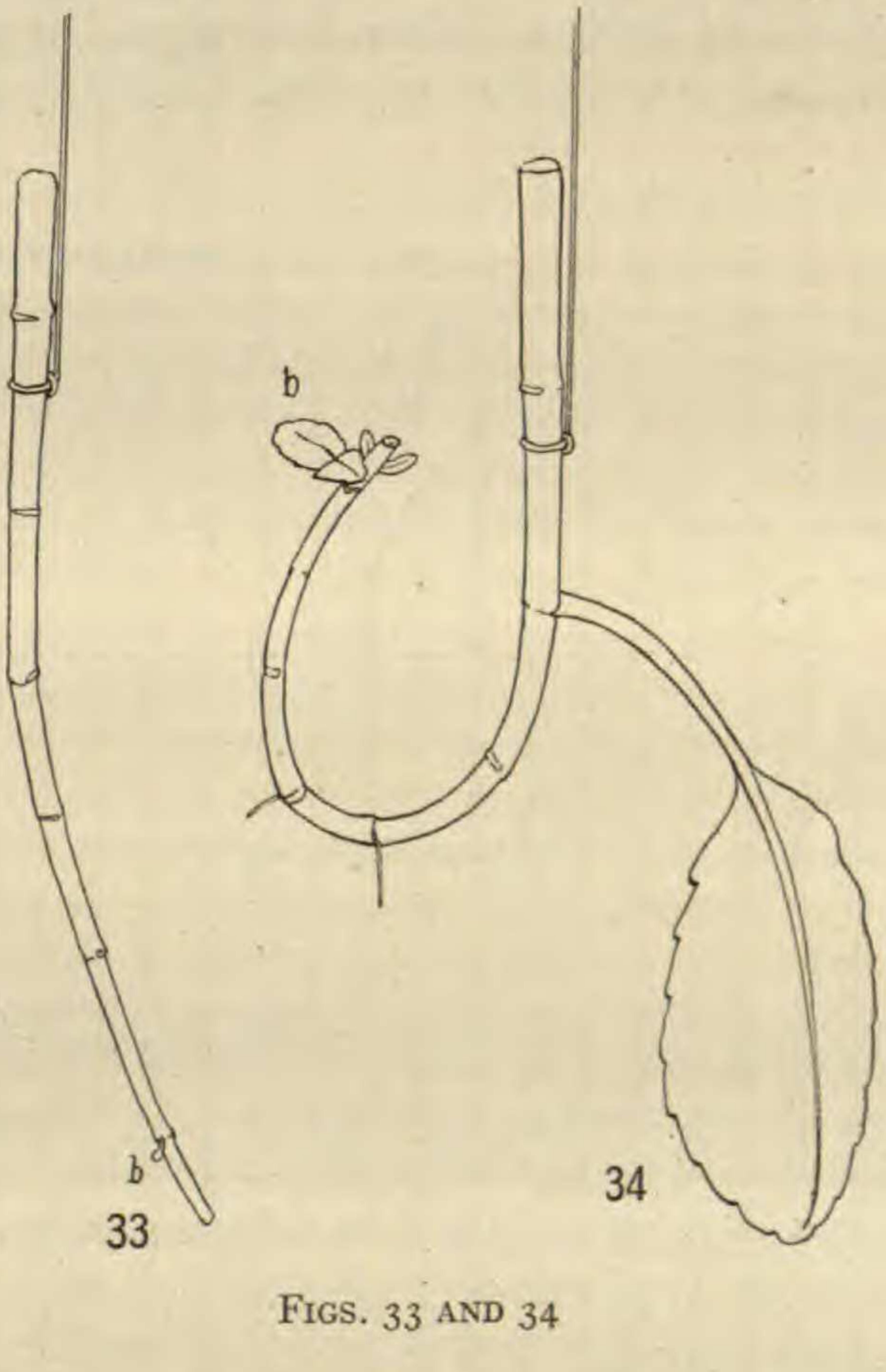
32

FIGS. 30–32

We will first show in a few simple examples that this idea leads us easily through the maze of facts in which the terms isolation or inhibition have no more than a metaphorical value.

When we isolate a leaf and suspend it in moist air or put it into a Petri dish the bottom of which is covered with water, as a rule only a few of the notches will grow out into shoots. Why do not all grow out? From what was said in the previous section it was natural to expect that the growth of the shoots in some of the notches of a leaf inhibits the growth in the rest of the notches of the same leaf, and that if all the notches could be isolated from each other this inhibiting effect would cease and they would all grow out. This idea was put to a test in a way indicated in fig. 37. Five notches on one side of a leaf were isolated from the leaf and from each other. The rest of the leaf and the isolated notches were put into a Petri dish whose bottom was covered with a layer of water. All five isolated notches grew out into shoots, while only three of the ten or twelve notches left on the leaf grew out. This experiment, which has often been repeated, succeeds easily if the piece of leaf left around each notch is not too small. It is noticeable that the rapidity of growth is greater in shoots which grow out from a whole leaf than in those growing out from the isolated notches. Here we see again an application of the rule that if an organ *a* inhibits the growth in *b*, the presence of *b* accelerates the growth in *a*. This is intelligible on the assumption that the leaf furnishes a flow of liquid containing material for the growth of shoots; and that the flow of this material away from the notches (wherever this may be) leads to the inhibition of the growth in the notches.

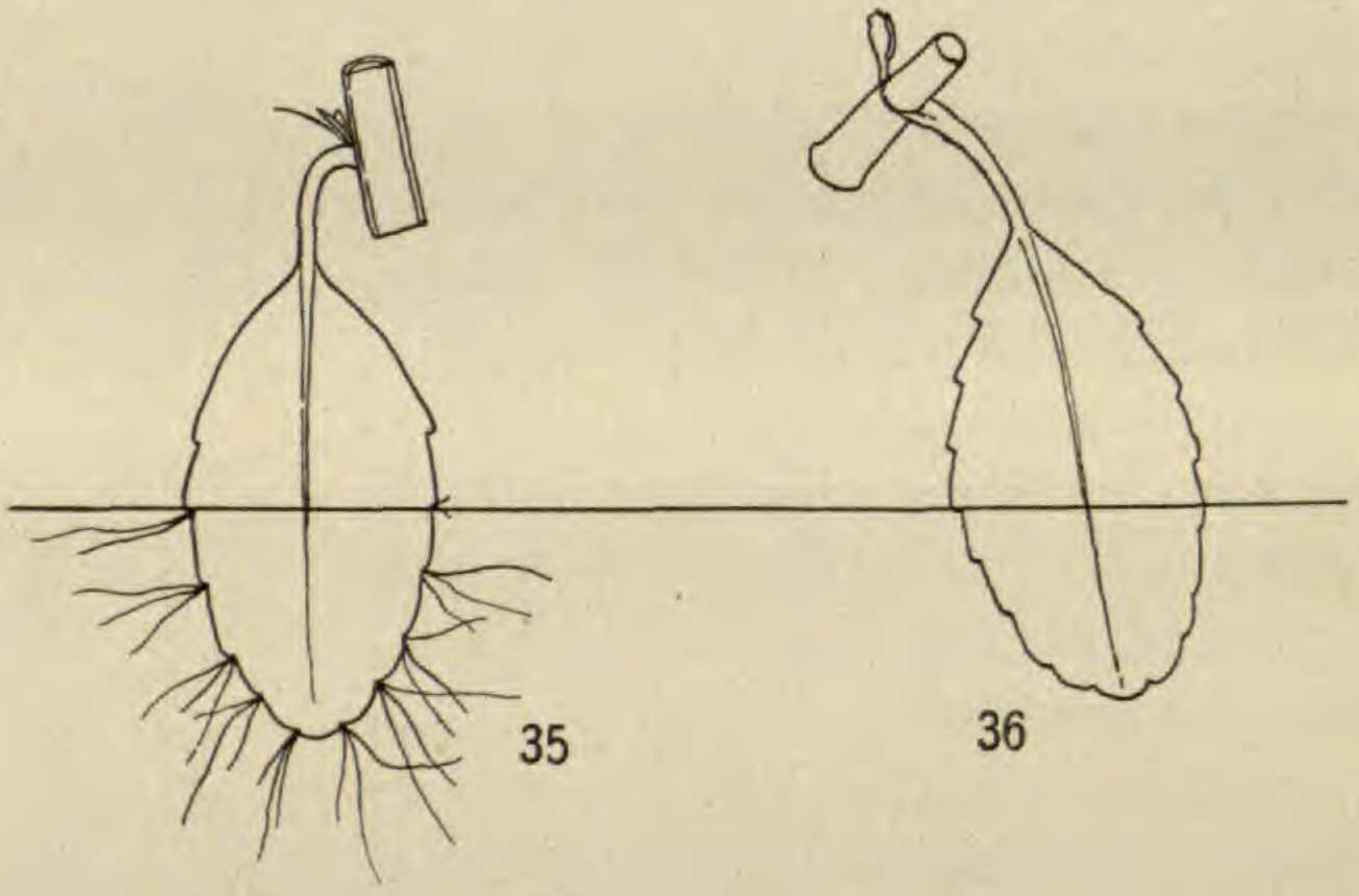
When the piece of leaf around an isolated notch is too small, no growth may occur or only tiny roots or shoots will grow out. This observation again agrees with the assumption that a notch of a leaf will grow into roots and shoots if certain substances or formed constituents of the leaf flow toward a notch or are prevented from flowing away.



FIGS. 33 AND 34

We can understand the experiment illustrated in fig. 37 on the assumption that if in a leaf one or more notches begin to grow out into roots and shoots, these shoots determine a flow in the rest of the leaf in a similar way as if a piece of the stem had remained attached to the leaf; and with the same inhibitory or retarding effect upon the growth of the other notches of the leaf. If, however, each notch is isolated and given enough water (for example, if it is put into a Petri dish which has a very thin layer of water), each notch can grow out, since the inhibitants through the establishment of currents in the leaf to growing shoots are lacking.

We have stated that if a leaf is suspended in moist air the growth of the shoots is prevented if a piece of a stem is left attached to the leaf. It seemed of interest to find out if this inhibiting effect would show itself even in a leaf in which a number of lateral incisions were made. This is indeed the case, as figs. 38 and 39 show. In these experiments the incisions were such that the pieces of the leaf had to be kept together by stitches of a thread. The leaves were suspended in moist air. Yet complete inhibition of the growth of



FIGS. 35 AND 36

the notches occurred in the leaf which was connected with a piece of stem (fig. 38). If a flow of substances from the leaf to the piece of stem is the cause of inhibition, such a flow must have taken place along a zigzag path in the leaf. One finds occasionally in such an experiment that in the extreme apical piece of the leaf the inhibiting effect of the stem may cease and that there a growth of roots may occur in the notches.

That the flow of water and of the material it carries in a stem may be deviated and altered by the growth of new shoots is rendered obvious by such observations as are represented in figs. 40 and 41. In this and similar cases thick pieces of the stem of *Bryophyllum* were cut out from the plant, deprived of their leaves, and put on moist soil. As is usual, shoots grew out very soon from the top buds of the stem; very soon afterward the piece in front of the top node began to shrink and wilt, not directly to the top node, but to within a few millimeters (fig. 40). When by chance the new shoot grows out not from the top node but from the one next to it, the whole piece in front of the top node may wilt (fig. 41).

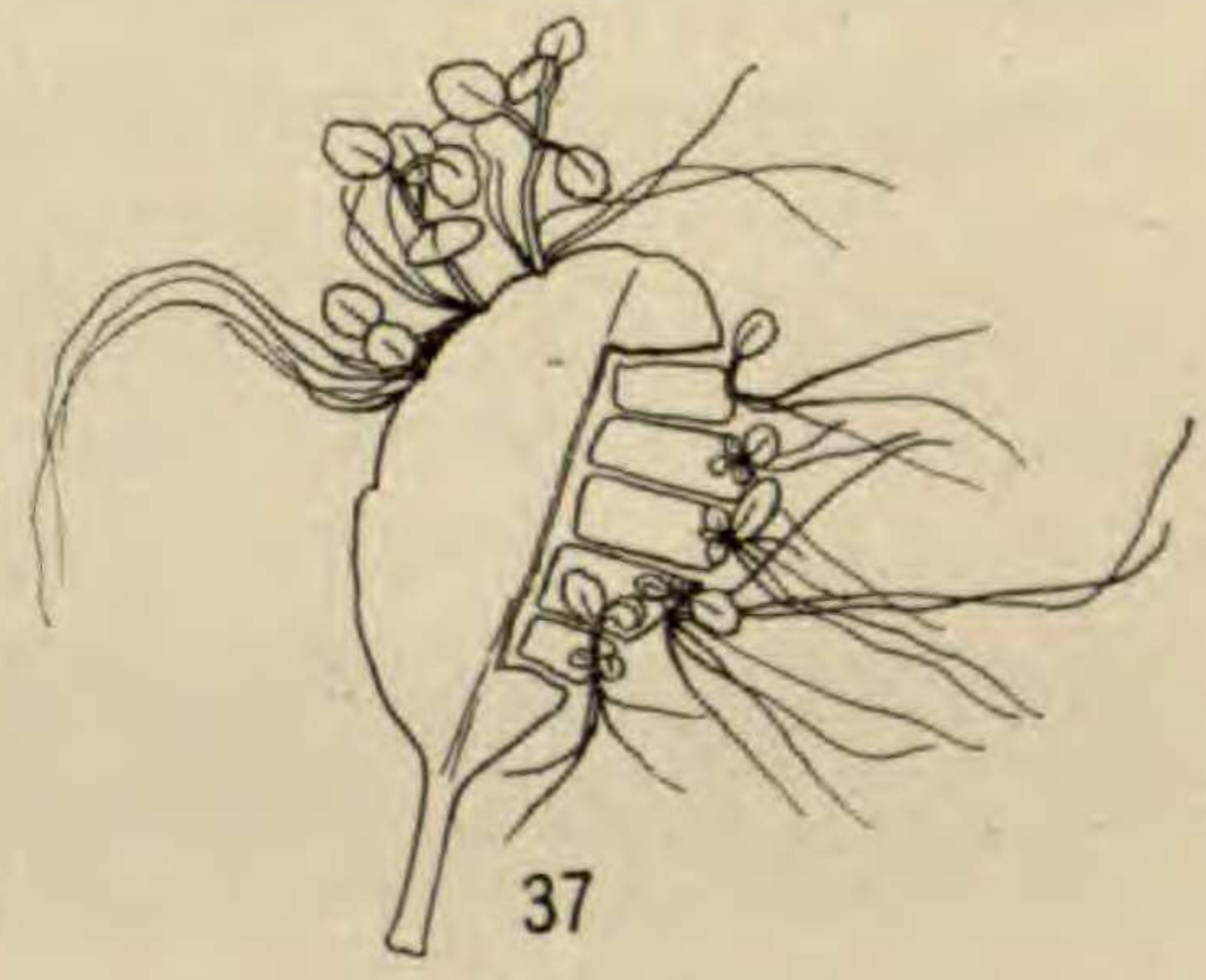
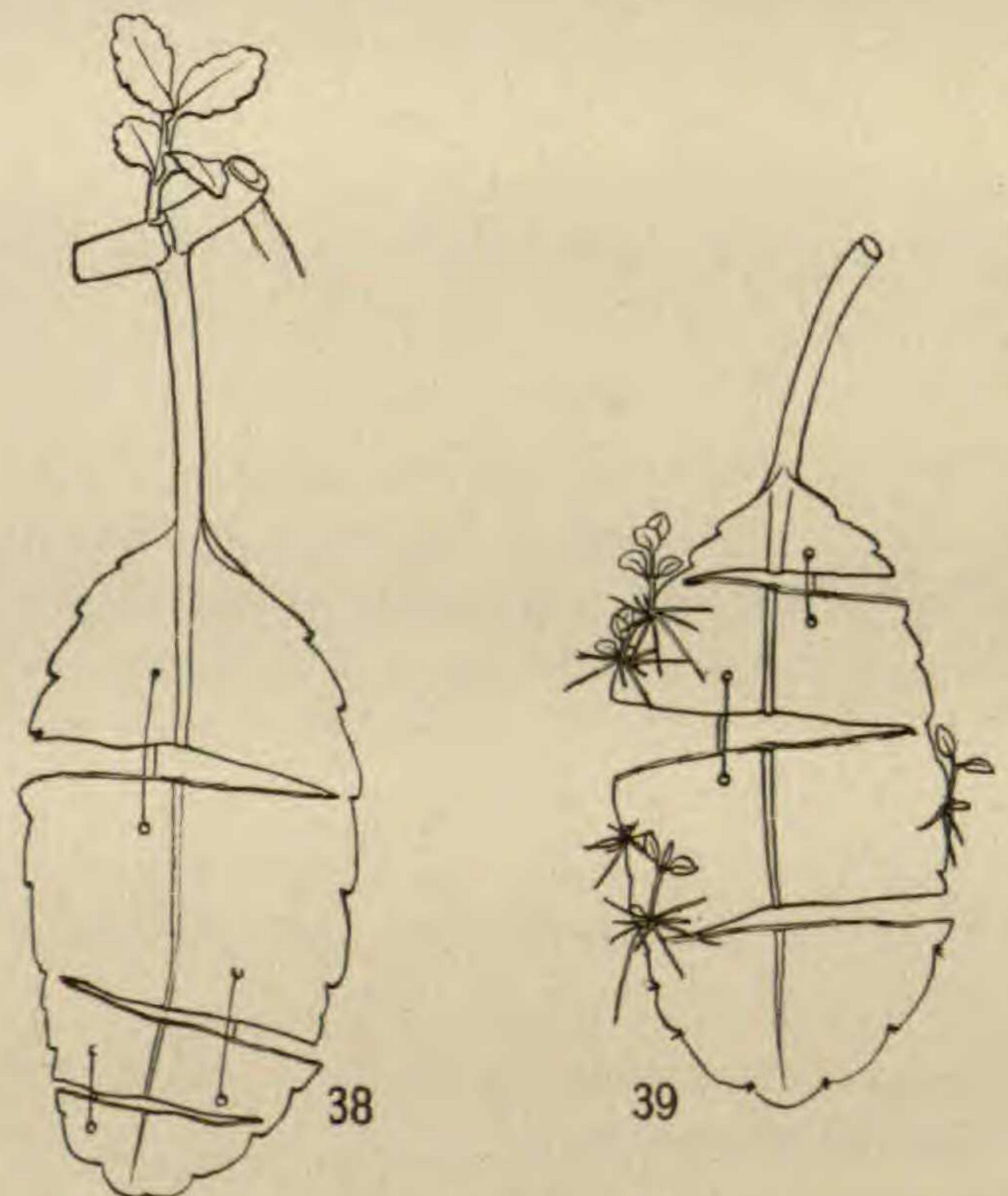


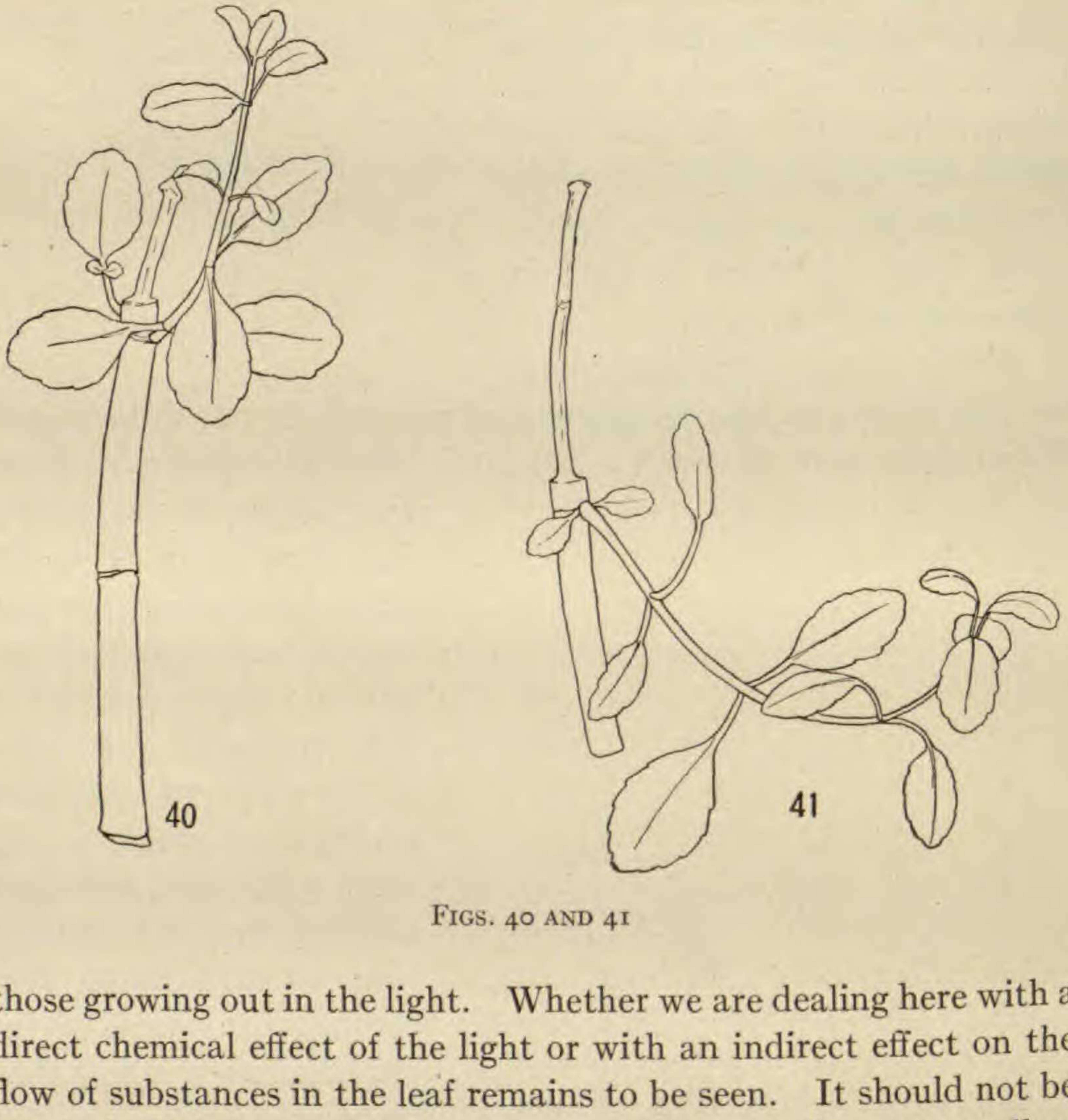
FIG. 37

These observations were made on stems kept in the laboratory rooms (not in the greenhouse). When the root of the stem is left intact and in its natural position, this wilting of the piece of the stem in front of the node from which the new shoots grow out will not occur. The ascending flow of liquid or material in the stem was deflected in this experiment into the most apical bud, and there was not enough root pressure to maintain a flow through the pieces of the old stem more apical than the new shoot.



FIGS. 38 AND 39

By way of parentheses we may here briefly mention that light exercises a great influence on the growth of the notches of an isolated leaf. If such leaves are suspended in moist air but free from light, as a rule none of the notches will grow out, while they will grow out promptly as soon as they are exposed to the light; provided they had not been kept too long in the dark. If the leaves are kept in the dark in a Petri dish whose bottom is covered with water, a few notches may grow out, but they are not nearly as numerous as



FIGS. 40 AND 41

those growing out in the light. Whether we are dealing here with a direct chemical effect of the light or with an indirect effect on the flow of substances in the leaf remains to be seen. It should not be overlooked that as soon as the leaves of *Bryophyllum* turn yellow they become less turgid and easily fall off from the stem.

X. Theoretical remarks

We may now go back to the first experiments mentioned in this paper and try to analyze them on the basis of the old idea that the flow of material in the plant is responsible for phenomena of growth. We start from the assumption that a notch of a leaf can grow out only if there is no flow of liquids (carrying non-formed or possibly formed material) away from the notches. This is not the case in the normal plant when the circulation is normal, and WAKKER and DEVRIES have shown the rôle which the root pressure plays in this case. But the root pressure is not the only factor which influences this flow. The experiments in figs. 1–3 seem to indicate that different factors aside from the root pressure can determine the flow away from the notches of the leaf, provided our assumption is correct that such a flow is the cause of the phenomena of growth and regeneration observed.

If we go back to these first experiments in this paper and try to formulate them in harmony with this idea, we should have to state that in a completely isolated leaf the flow away from the notches ceases. As a consequence, one or more of the notches may grow out, and as soon as this happens the flow in the leaf is directed toward the growing notches. They act as if they exercised a "suction" on the flow of liquids in the leaf, and they may inhibit the growth in other notches of the leaf.

If the leaf is in connection with a piece of stem, the latter exercises this "suction," and the flow of liquids is away from the leaf to the stem; hence the inhibiting effect of the stem upon the growth of the notches of the leaf. This "suction effect" is especially great if the bud opposite this leaf can grow out, as in fig. 2. If both leaves are left attached to the piece of stem (as in fig. 3), the flow from a leaf will be deflected from the buds and may go into the opposite leaf. This might explain why when both leaves remain attached to a piece of stem the growth of the notches of the leaves is favored again, though it is not so rapid as in a completely isolated leaf.

This idea of a deflection of the current away from the leaf toward the opposite side of the stem is in harmony with the fact that the bud opposite a leaf grows out very quickly if its own leaf is removed (fig. 2); while the growth of the axillary bud of the leaf which is not removed is inhibited in this case. If we split the stem longitudinally, this deflection ceases and the leaf ceases to inhibit the growth of its own axillary bud. This idea is supported by the fact that if the leaf attached to a longitudinally split node is partly suspended in water its notches will grow out rather rapidly.

We have assumed that if we have a node with its two leaves attached, the flow will be deflected from the buds; this again is in harmony with the facts that in such a case as a rule neither of the buds grows out.

When a plant is normal, it is almost or possibly absolutely impossible to induce the notches of a leaf which is connected with the plant to grow. The writer has submersed such leaves in water, but in months not a single notch ever formed a root or a shoot. If, however, the flow of substances in a plant is abnormal, either because the roots or the apical parts or both have suffered, a growth of shoots may occur in moist air from the notches of leaves which are in contact with the plant. This fact is mentioned by DeVries and is well known to those who have seen the plants in their natural conditions in Bermuda.

If we now return to the question from which we started, namely, why it is that the notches of the leaves of *Bryophyllum* will not grow out while in connection with the normal plant, the answer should be that the flow of material from the root and from the leaves into the stem and to the apical end of the latter prevents this growth. Through this flow material is carried away from the notches of the leaves. The anatomy of the conducting vessels and tissues, which is inherited, and the dynamical factors determining the flow are the factors concealed in the term "correlation." We understand why it is that if we isolate a part, buds may grow out which without the isolation would not have grown, the reason being that in the mutilated part material can flow to and be retained at places where if the part had remained in the whole it could not have been retained. This assumption agrees with the older ideas of Dutrochet, Sachs, DeVries, and Goebel on regeneration in plants. We understand on this basis why it is that the term isolation of parts or the inhibiting effect of growing parts on others may express some but not all the facts of regeneration. It is not the isolation in itself, but the retention of material in places where there would not have been such a retention under ordinary conditions which apparently determines the growth of dormant buds in an isolated piece; and so it may happen that while this term expresses adequately some results, it fails in others.

SACHS assumed that specific organ-forming substances were needed for growth, and that the consumption of these substances in the growing regions was the cause of the inhibition of growth in the dormant buds of a plant. While the first half of the theory may be correct, the second part is not tenable, since in each stem of a *Bryophyllum* there is enough "formative" material to allow each bud in all the nodes to grow out, while as a matter of fact only the most apical ones will do so. This is intelligible on the assumption that these apical nodes retain the "formative" material in excess of what they need for their own growth.

The ideas expressed in this paper agree in the main with the results and conclusion of the author's older experiments on regeneration in animals. The writer had found that if a piece be cut out from a stem of a *Tubularia* a new polyp may form at each end of the stem, but that the formation of the polyp at the oral end precedes that at the aboral end; and the difference in time may be from one or two days to as many weeks, according to the species or the temperature. He found also that the formation of the oral polyp is the cause of the delay in the formation of the aboral polyp, and that if he prevented the formation of the oral polyp this delay in the formation of the aboral polyp was no longer observed.[7] This is the same rule which we have found for the relation between the growth of the bud of the stem and the formation of adventitious roots and shoots in the opposite leaf of *Bryophyllum*. The growth of this bud causes a delay in the growth of adventitious roots and shoots in the opposite leaf, and this delay is suppressed or diminished if the bud is prevented from growing.

The writer suggested that a flow of substances was the cause of these phenomena of correlation in *Tubularia*. He had found that pigmented cells which come from the entoderm and are carried in the circulation are always collected at the spot where regeneration of the natural growth of the hydroid is to start.

These remarks may suffice to indicate that the rules of inhibition observed in *Bryophyllum* may have a wider application.

[7] LOEB, J., Untersuchungen zur physiologischen Morphologie der Tiere. I and II. Würzburg. 1890 and 1891; Pflüger's Archiv **102**:152. 1904.

XI. Summary of results

The phenomena of inhibition of regeneration have been studied in *Bryophyllum calycinum* and it was found that they are governed by the following simple rule:

If an organ *a* inhibits the regeneration or growth in an organ *b*, the organ *b* often accelerates and favors the regeneration in *a*.

This rule is best understood on the assumption that the inhibiting organ receives something from the inhibited organ necessary for regeneration.

It is pointed out that this harmonizes with the older assumption of botanists and of the writer that the flow of material and the block to such a flow after mutilation is responsible for the phenomena of inhibition in regeneration, as well as for the phenomena of correlation.

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